CLAIMS

1. A method for identifying an essential gene of an organism comprising:

(i) providing a library of transposon mutants of the said organism;

(ii) isolating polynucleotide sequences from the library which flank inserted transposons;

(iii) hybridising the said polynucleotide sequences with a polynucleotide library from the said organism; and

(iv) identifying a polynucleotide in the said polynucleotide library to which the said polynucleotide sequences do not hybridise, thereby to determine an essential gene of the organism.

2. A method according to claim 1, wherein the said polynucleotide library is in the form of a gridded array.

3. A method according to claim 1 or 2, wherein the organism is a bacterium, yeast, fungus, plant or animal.

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4. A method according to any one of the preceding claims, wherein:

in step (ii) polynucleotide sequences flanking one side of the transposons are isolated to give a pool of sequences and polynucleotide sequences flanking the other side of the transposons are isolated to give a separate second pool of sequences; and

in step (iii) the first pool of sequences is hybridised with a first sample of the said polynucleotide library and the second pool of sequences is hybridised with a second sample of the said polynucleotide library.

5. A method according to claim 4, wherein in step (ii) each pool of sequences is isolated by a method comprising:

(a) digesting genomic DNA isolated from a library of transposon-tagged mutants with a restriction endonuclease that cuts within the transposon (T-specific endonuclease) and a second different

restriction endonuclease (G-specific endonuclease) which cuts within the disrupted sequence;

(b) ligating the resulting DNA fragments with a linker;

(c) carrying out PCR on the resulting DNA fragments with an oligonucleotide specific for a transposon sequence and an oligonucleotide specific for a linker sequence.

6. A method according to claim 4 or 5, wherein the library of transposon mutants is a library of Tn*phoA* *E. coli* mutants.

7. A method according to claim 6, wherein:

in the isolation of the first pool of sequences the restriction enzyme which cuts in the transposon is *Dra*I and the second enzyme is a 4 base pair restriction endonuclease; and

in the isolation of the second pool of sequences the restriction enzyme which cuts in the transposon is *Hpa*I and the second enzyme is a 4 base pair restriction endonuclease.

8. A method for identifying a conditional essential gene of an organism comprising:

(i) providing a first sample of a library of transposon mutants of the said organism (input library);

(ii) providing a second sample of the library and subjecting that sample to a conditional restraint;

(iii) collecting the mutants that survive the conditional restraint in step (ii) to give a new library (output library);

(iv) carrying out a method according to any one of the preceding claims on the input library from step (i) and on the output library from step (iii), thereby to determine a conditional essential gene of the organism.

9. Use of an essential gene identified by a method according to any one of

claims 1 to 7 or a conditional essential gene according to claim 8, or the polypeptide encoded by a said gene, in a method for identifying an inhibitor of transcription and/or translation of that gene and/or activity of a polypeptide encoded by that gene.

10. A method for identifying:

(i) an inhibitor of transcription and/or translation of an essential gene identified by a method according to any one of claims 1 to 7 or a conditional essential gene identified by a method according to claim 8; and/or

(ii) an inhibitor of activity of a polypeptide encoded by a said gene,

which method comprises determining whether a test substance can inhibit transcription and/or translation of a said gene and/or activity of a polypeptide encoded by a said gene.

11. An inhibitor identified by a method according to claim 10.

12. An inhibitor of transcription and/or translation of an essential or conditional essential gene and/or activity of a polypeptide encoded by that gene.

13. An inhibitor according to claim 11 or 12, wherein the essential or conditional essential gene is a bacterial, fungal or eukaryotic parasite essential or conditional essential gene.

14. An inhibitor according to claim 13 for use in a method of treatment of the human or animal body by therapy.

15. An inhibitor according to claim 14 for use in a method of treatment of a bacterial, fungal or eukaryotic parasite infection.

16. Use of an inhibitor according to claim 13 for the manufacture of a medicament for use in the treatment of a bacterial, fungal or eukaryotic parasite

infection.

17. A pharmaceutical composition comprising an inhibitor according to claim 13 and a pharmaceutically acceptable carrier or diluent.

18. A method of treating a host suffering from a bacterial, fungal or eukaryotic parasite infection, which comprises administering to the host a therapeutically effective amount of an inhibitor according to claim 13.

19. An inhibitor according to claim 11 or 12, wherein the essential or conditional essential gene is a bacterial, fungal or pest essential or conditional essential gene.

20. Use of an inhibitor according to 19 as a plant bactericide, fungicide or pesticide.

21. An inhibitor according to claim 11 or 12, wherein essential or conditional essential gene is a plant conditional or essential gene.

22. Use of an inhibitor according to claim 21 as a herbicide.

23. A method according to claim 8, wherein the organism is a bacterium and the conditional restraint is growth of that bacterium in its host.

24. A bacterium attenuated by a non-reverting mutation in one or more genes identified by a method as defined in claim 23.

25. A vaccine comprising a bacterium according to claim 24 and a pharmaceutically acceptable carrier or diluent.

26. A bacterium according to claim 24 for use in a method of vaccinating a human or animal.

27. Use of a bacterium according to claim 24 for the manufacture of a medicament for vaccinating a human or animal.

28. A method of raising an immune response in a mammalian host, which comprises administering to the host a bacterium according to claim 24.